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Note

Thin-layer chromatographic detection of the herbicide asulam in soils and the identification of sulphanilamide as a minor soil degradation product

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The herbicide asulam (methyl 4-aminobenzenesulphonylcarbamate) is extensively used for the control of various annual and perennial weeds in a variety of crops.

The herbicide undergoes rapid degradation in soils over a range of temperature and moisture conditions[1–3], and a *Flavobacterium* species has been isolated[4] from asulam-treated soil capable of growing with the herbicide as carbon and energy source.

In the absence of radioactively-labelled asulam, the isolation of degradation products from the soil is difficult. Previous investigations[2,3] have relied upon thin-layer chromatographic (TLC) separation of the coloured complexes formed from soil extracts which were first diazotised and then treated with Marshall's reagent [N-(1-naphthyl)ethylenediamine dihydrochloride][6]. Under such conditions, primary amines form red complexes which can be solvent extracted and subjected to TLC analysis. It has been postulated that sulphanilamide and sulphanilic acid may be formed in soil by degradation of the parent herbicide[3]. Although it has been noted[5] that asulam adsorbed onto montmorillonite can be converted to sulphanilic acid when heated, to date no degradation products have been reported from soils treated with asulam.

Thin-layer chromatographic separation of asulam, sulphanilic acid and sulphanilamide has been described[7], with visualisation being effected by observing the developed chromatograms under shortwave ultraviolet wavelengths. However this procedure is not sufficiently sensitive to detect minute amounts of amide and acid that could be formed in the soil.

In the work to be described, a TLC procedure was developed to monitor the degradation of asulam in three Saskatchewan soils and determine whether sulphanilamide and sulphanilic acid were being formed as degradation products. Fluorescamine was used as the chromogenic spray since primary amino groups, including those of 4-aminosulphonamides, readily react with this reagent[8,9] to form highly fluorescent derivatives, thus allowing the detection of minute amounts of such compounds.

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EXPERIMENTAL

Chemicals

Pure asulam was provided by May & Baker (Dagenham, Great Britain) while the sulphanilamide and sulphanilic acid were obtained from Eastman-Kodak (Rochester, NY, U.S.A.). Standard solutions of asulam and sulphanilamide were prepared in methanol at concentrations of 10 μg/ml, while a solution of sulphanilic acid was prepared containing 10 μg/ml of water.

Thin-layer plates

Precoated TLC plates (silica gel 60F-254) were obtained from E. Merck (Darmstadt, G.F.R.). Following development to a height of 10 cm above the origin the plates were air-dried and sprayed with chromogenic reagent. The R_F values of asulam, sulphanilamide and sulphanilic acid in two selected chromatographic solvent systems are shown in Table I.

Chromogenic reagent

Fluorescamine (12 mg), obtained from Aldrich (Milwaukee, WI, U.S.A.), was dissolved in acetone (100 ml) and sprayed onto the developed plates in a darkened room. After a 20 min period in the dark, the plates were viewed under longwave ultraviolet wavelengths when asulam, sulphanilamide and sulphanilic acid appeared as fluorescent spots on a purple background.

Soil studies

Soils. Samples of a clay loam, heavy clay and sandy loam were collected from the surface 10-cm horizon during September 1982. The soils were screened through a 2-mm sieve and stored in plastic sacks at room temperature until January 1983 when the studies were commenced. The composition and physical characteristics of these soils have already been reported[10].

Persistence studies. The experimental design was very similar to that described earlier for persistence studies with asulam[3], with samples (50 g) of the three soils at 85% of their field capacity moistures being treated with 50-μl portions (500 μg asulam) of a solution containing 10 mg herbicide per ml methanol. All treatments were incubated in the dark at 20°C, with samples being extracted and analysed for degradation products after 4, 8, 14 and 21 days.

Soil extraction and analysis. The soils were placed in 250-ml glass-stoppered flasks and methanol added so that the volume of methanol together with the water present in the soil was equal to 100 ml. The flasks were shaken on a wrist-action shaker for 1 h. Following centrifugation at 2000 *g* for 5 min, 10 μl supernatant from each soil extract were carefully applied to the origin of the chromatographic plates to assess the presence of asulam, while 100-μl portions were similarly applied to determine the presence of sulphanilamide and sulphanilic acid. In all instances 50-, 40-, 30-, 20- and 10-ng portions of asulam, sulphanilamide and sulphanilic acid were applied to the plate origins as reference compounds. After development and treatment with chromogenic spray, the intensities of the fluorescent spots from both soil extracts and standards were compared visually to give a qualitative assessment of the amounts of the three compounds recovered from the soils.

Air-dried samples of all three soils were fortified at the 10 and 1 ppm levels with asulam, sulphanilamide and sulphanilic acid. After a 24-h equilibration period, the soils were extracted as described when 10- and 100-μl portions of the methanolic extracts were subjected to TLC analysis. Recoveries of all compounds from all soils were over 80%. Prior experiments also confirmed that no interfering substances were present in methanolic extracts derived from untreated soils, and that the asulam used in these studies contained no detectable traces of sulphanilamide or sulphanilic acid.

RESULTS AND DISCUSSION

The chromogenic spray fluorescamine allows 10-ng amounts of asulam, sulphanilamide and sulphanilic acid to be observed on the developed chromatograms and is thus an extremely sensitive reagent for these compounds. Furthermore, the intensity of the fluorescent spots increased with increasing concentration of primary amine applied to the TLC plate so that qualitative assessment of the amounts of the three compounds extracted was easily achieved. The simple methanolic extraction therefore permits the detection of all three chemicals in the soil at the 0.2 ppm level without resort to concentration and clean-up procedures. The different R_F values resulting from the two solvent systems (Table I) provide both identification and necessary confirmation for asulam, sulphanilamide and sulphanilic acid.

TABLE I

R_F VALUES OF COMPOUNDS STUDIED

Solvents: I = chloroform–methanol (1:1); II = glacial acetic acid–methanol–benzene (1:1:3).

Compound	R_F	
	I	*II*
Asulam	0.85	0.78
Sulphanilamide	0.77	0.63
Sulphanilic acid	0.38	0.21

Although residues of asulam normally to be expected in the soil following spray treatments are of the order of 2 ppm[3], the higher rate of 10 ppm was chosen in these studies to enable small amounts of the possible degradation products to be detected.

The results from the persistence studies (Table II) indicate that in all three soils asulam underwent a rapid degradation. In all cases the time required for 50% of the asulam to be degraded was approximately 7 days. This half-life value for asulam in the heavy clay is similar to that reported earlier for this soil[3].

No traces of sulphanilic acid were detected in any of the soil extracts, whereas sulphanilamide was recovered from all of the soils at all sampling dates (Table II). The amounts of sulphanilamide detected were dependent upon soil type with the least amounts being formed in the heavy clay and the greatest amounts being isolated from the clay loam (Table II).

The present study thus indicates that sulphanilamide is formed in soils as a

TABLE II

BREAKDOWN OF 10 PPM ASULAM IN A CLAY LOAM (CL), HEAVY CLAY (HvC) AND SANDY LOAM (SL) AT 20°C AND 85% OF FIELD CAPACITY

Time (days)	*Amount recovered (ppm)*					
	Asulam			*Sulphanilamide*		
	CL	*HvC*	*SL*	*CL*	*HvC*	*SL*
4	6	6	6	0.4	<0.2	0.2
8	4	4	4	0.8	<0.2	0.2
14	2	4	2	0.8	0.2	0.3
21	1	3	1	0.8	0.3	0.3

result of asulam degradation. Although the amounts formed appear to be dependent upon soil type, less than 10% of the applied herbicide was converted to sulphanilamide in the soils studied. Previous work[3] has shown that sulphanilamide is degraded in moist soils with a half-life value similar to that for asulam.

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